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Extension-Trapping SNP Assay

Highly Stringent Annealing Conditions (gDNA is biotinylated prior to assay)

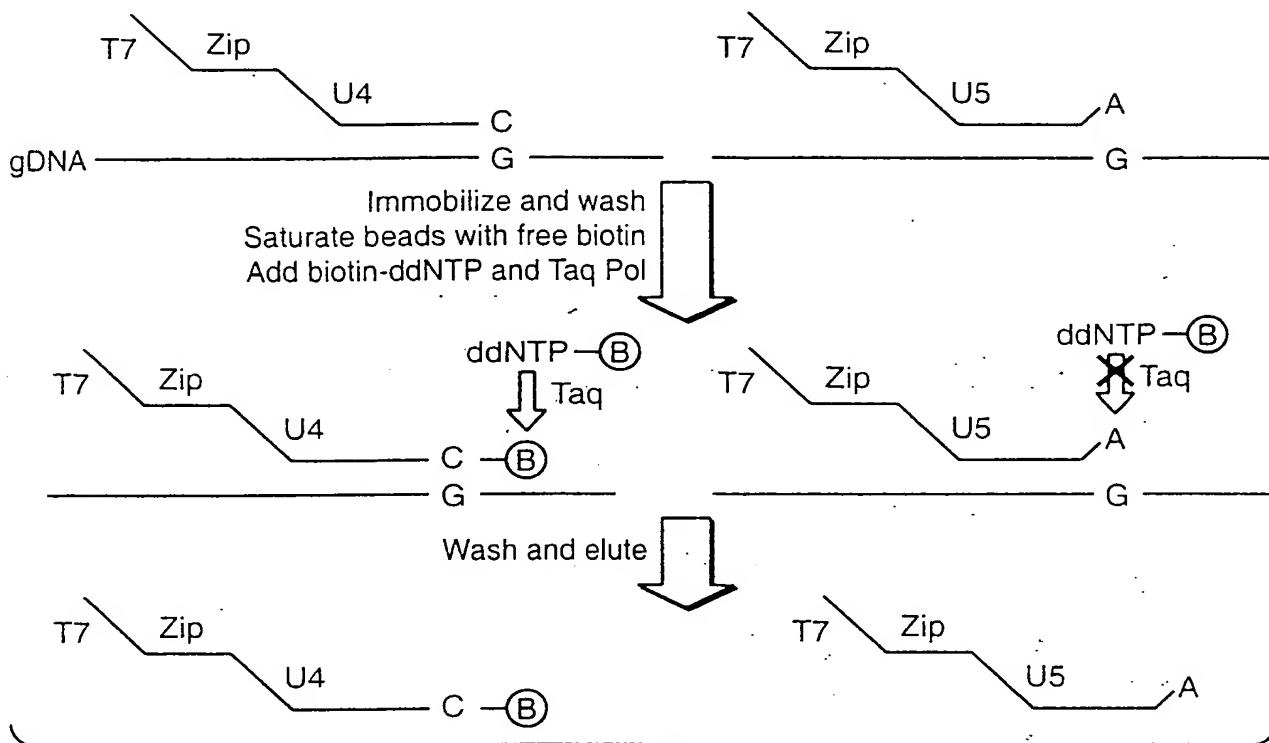


FIG. 1A

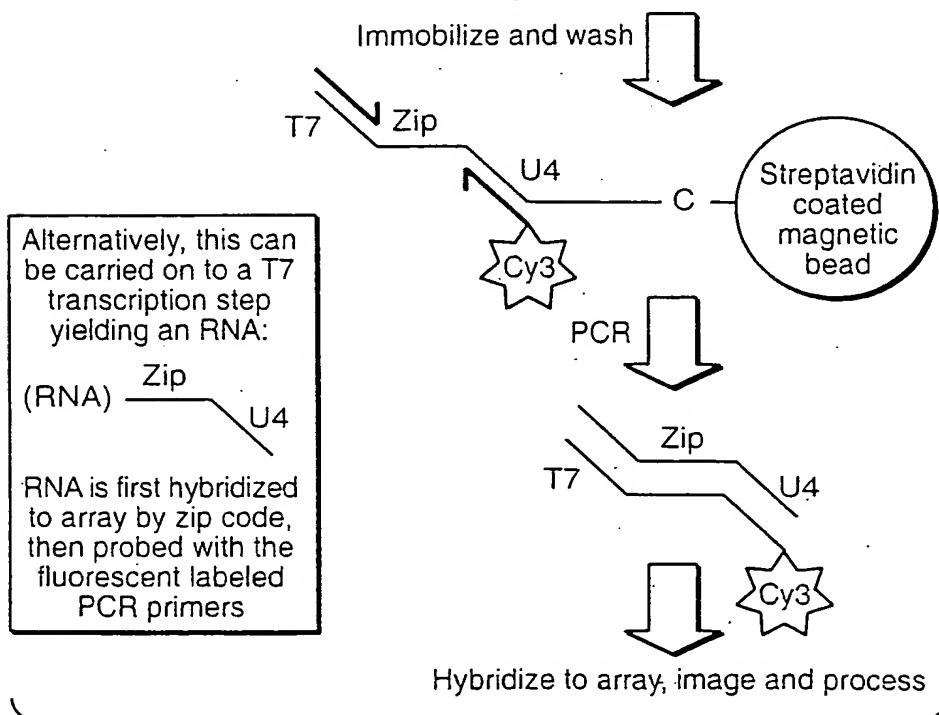


FIG. 1B

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Reduced Genome Single Base Extension Assay

Stringent Annealing Capture and Wash

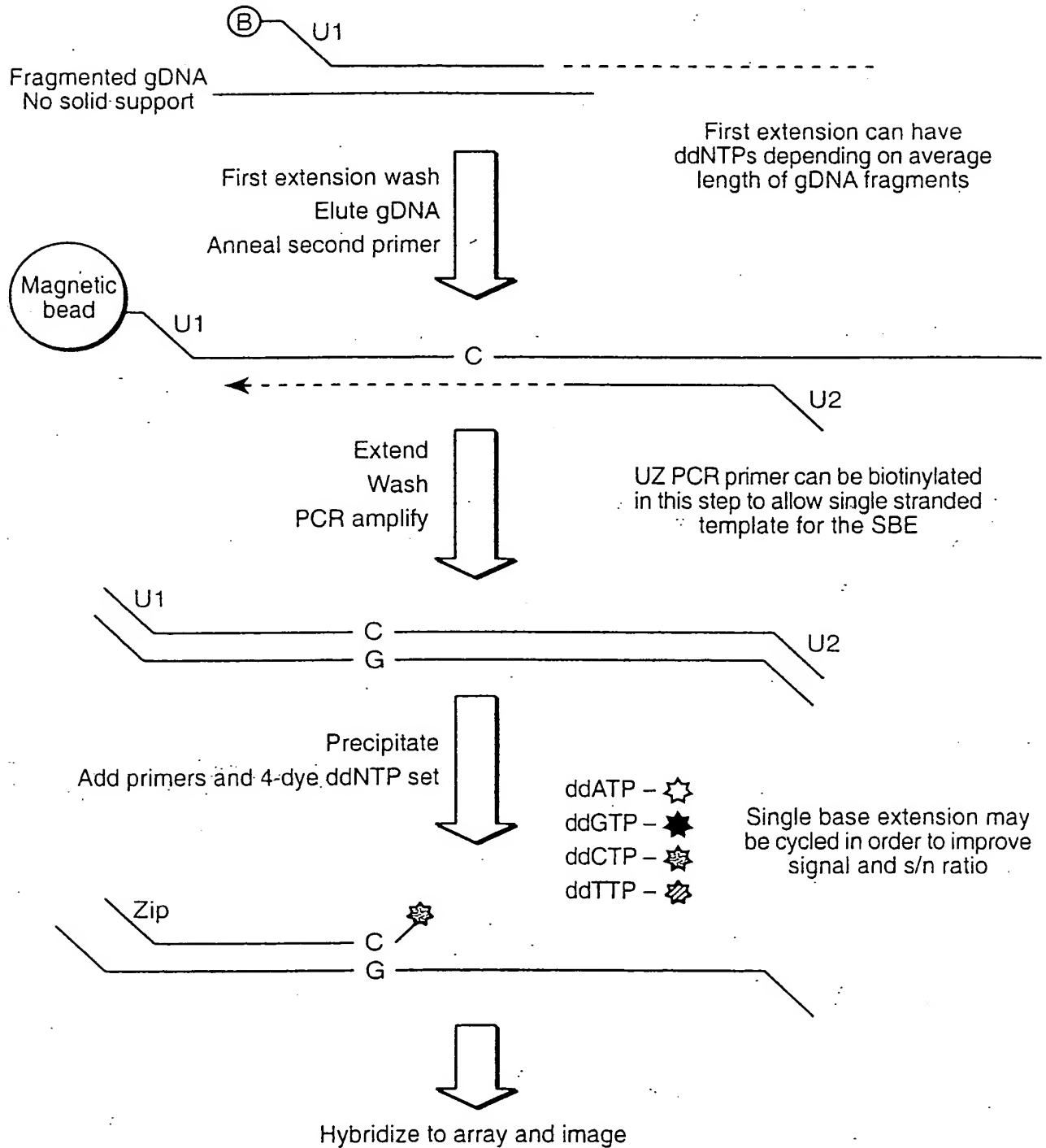


FIG. 2

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Complexity Reduction and Multiplex Assay

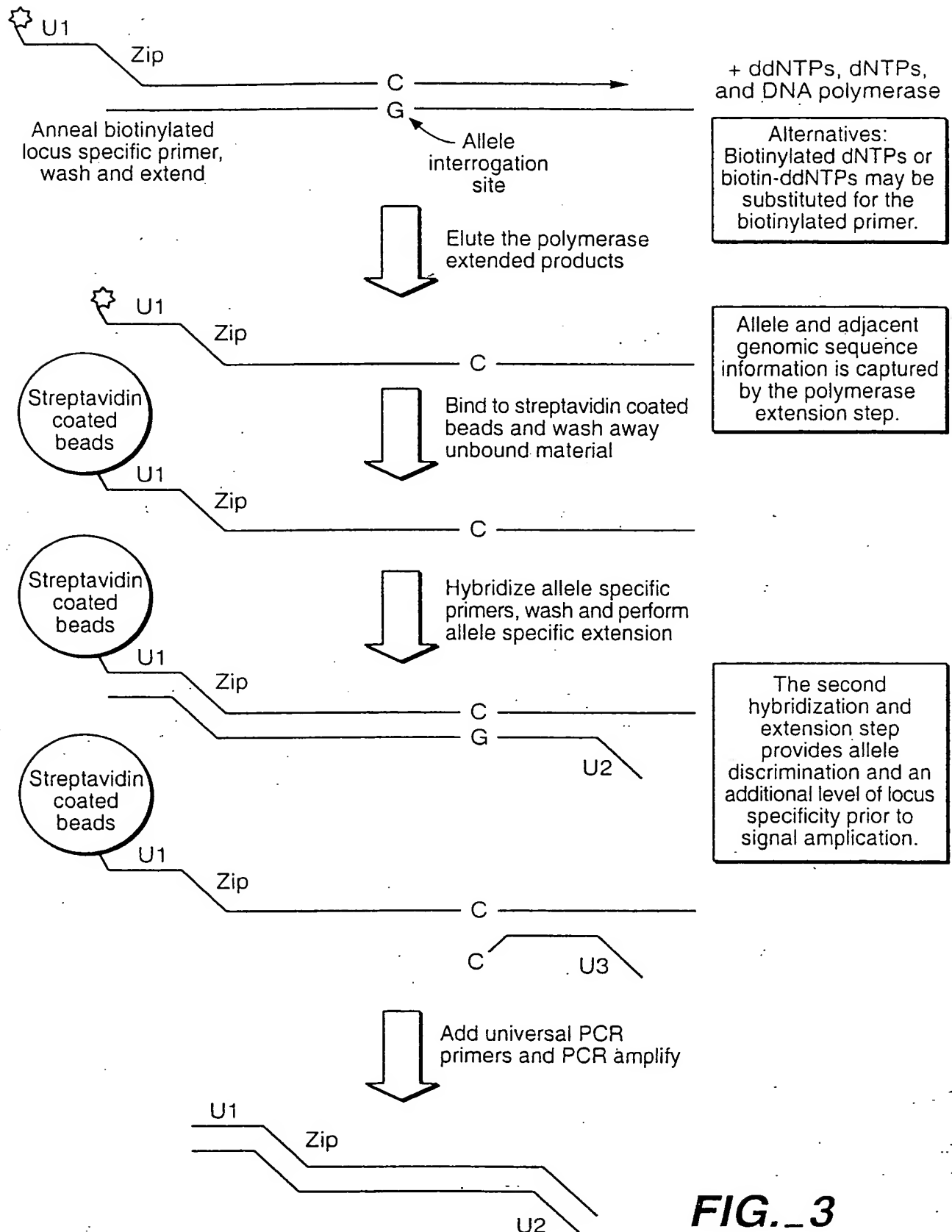
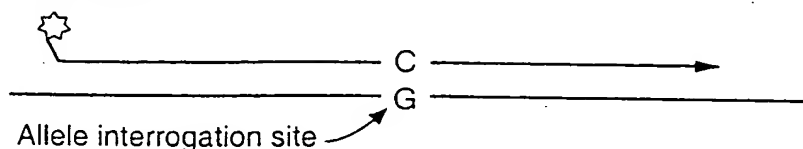


FIG._3

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Complexity Reduction and Multiplex Assay

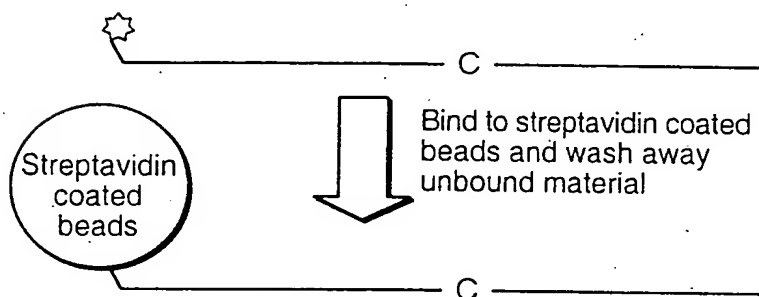
Anneal biotinylated
locus specific primer,
wash and extend



+ ddNTPs, dNTPs,
and DNA polymerase

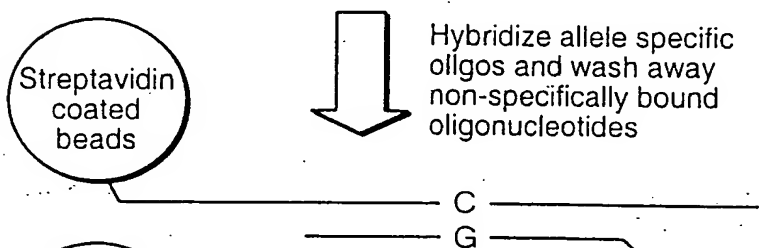
Alternatives:
Biotinylated dNTPs or
biotin-ddNTPs may be
substituted for the
biotinylated primer.

Elute the polymerase
extended products



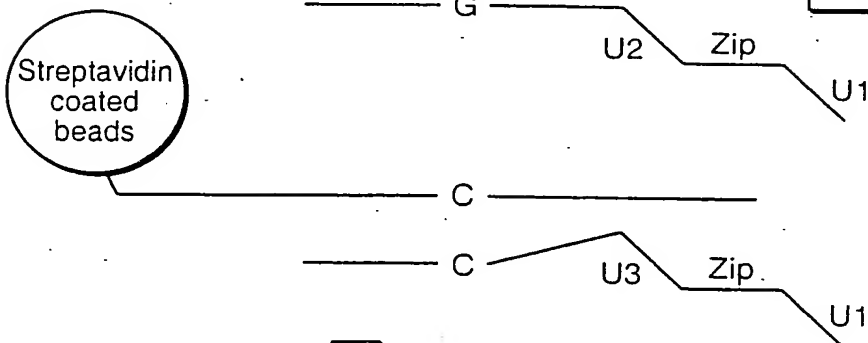
Allele and adjacent
genomic sequence
information is captured
by the polymerase
extension step.

Bind to streptavidin coated
beads and wash away
unbound material



The second
hybridization
step provides allele
discrimination and an
additional level of locus
specificity prior to
signal amplification.

Hybridize allele specific
oligos and wash away
non-specifically bound
oligonucleotides



Add universal PCR
primers and PCR amplify

This oligonucleotide is
washed away under
stringent hybridization
and wash conditions.

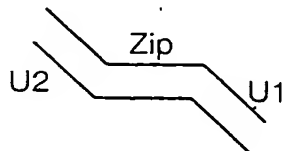


FIG. 4

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Complexity Reduction and Multiplex Assay

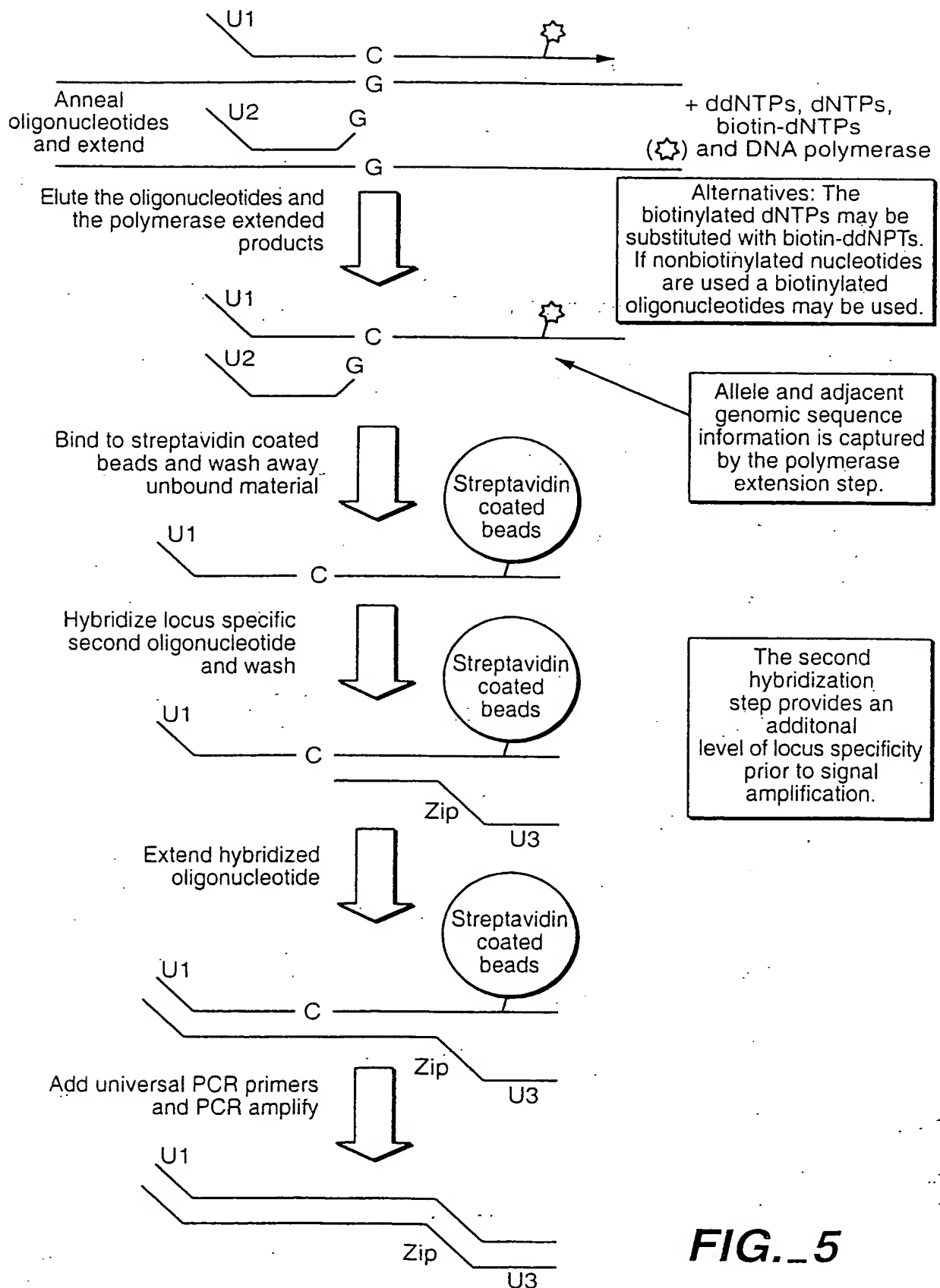


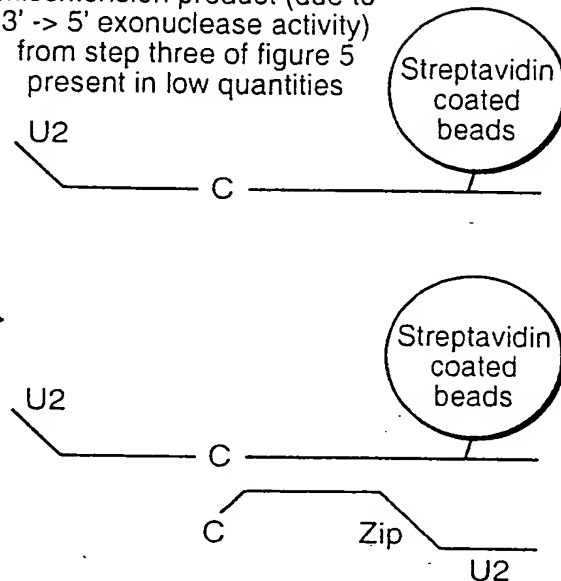
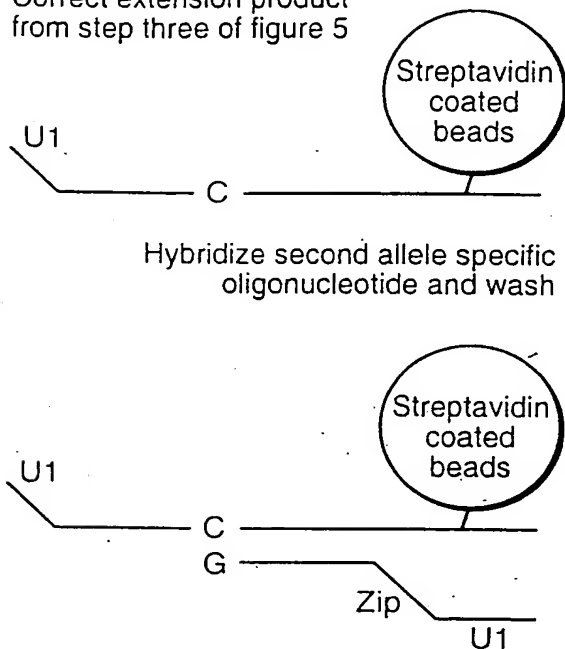
FIG. 5

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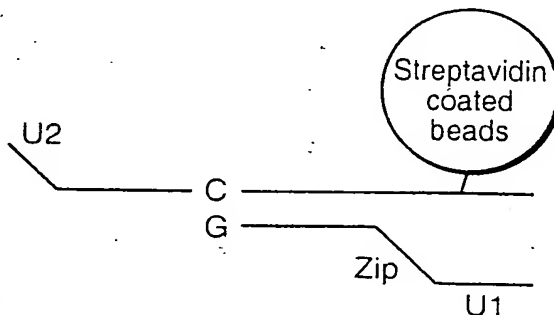
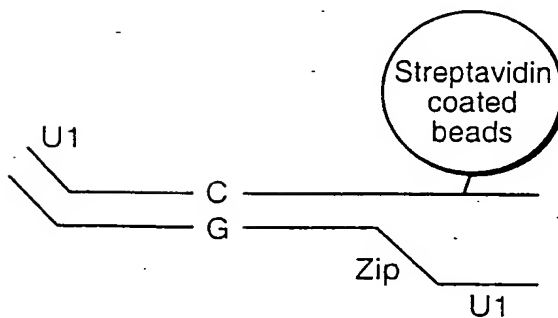
Complexity Reduction and Multiplex Assay

Correct extension product
from step three of figure 5

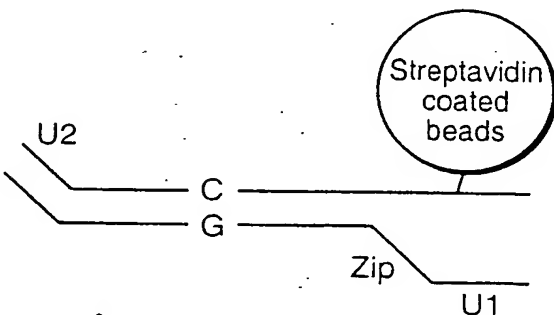
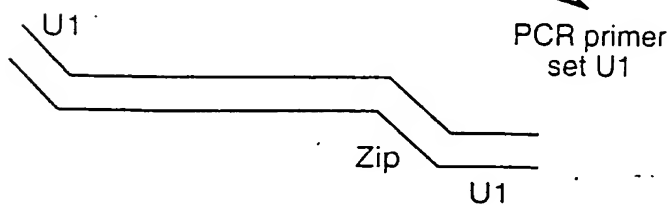
Misextension product (due to
3' -> 5' exonuclease activity)
from step three of figure 5
present in low quantities



Extend correctly hybridized
oligonucleotides



Split extension reaction and
add to universal PCR primer
set U1 and set U2



X
No amplification

FIG._6

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Solid Phase Locus-Specific Primer Extension

Starting material is immobilized, single stranded universal PCR product.
There are several ways to generate this.

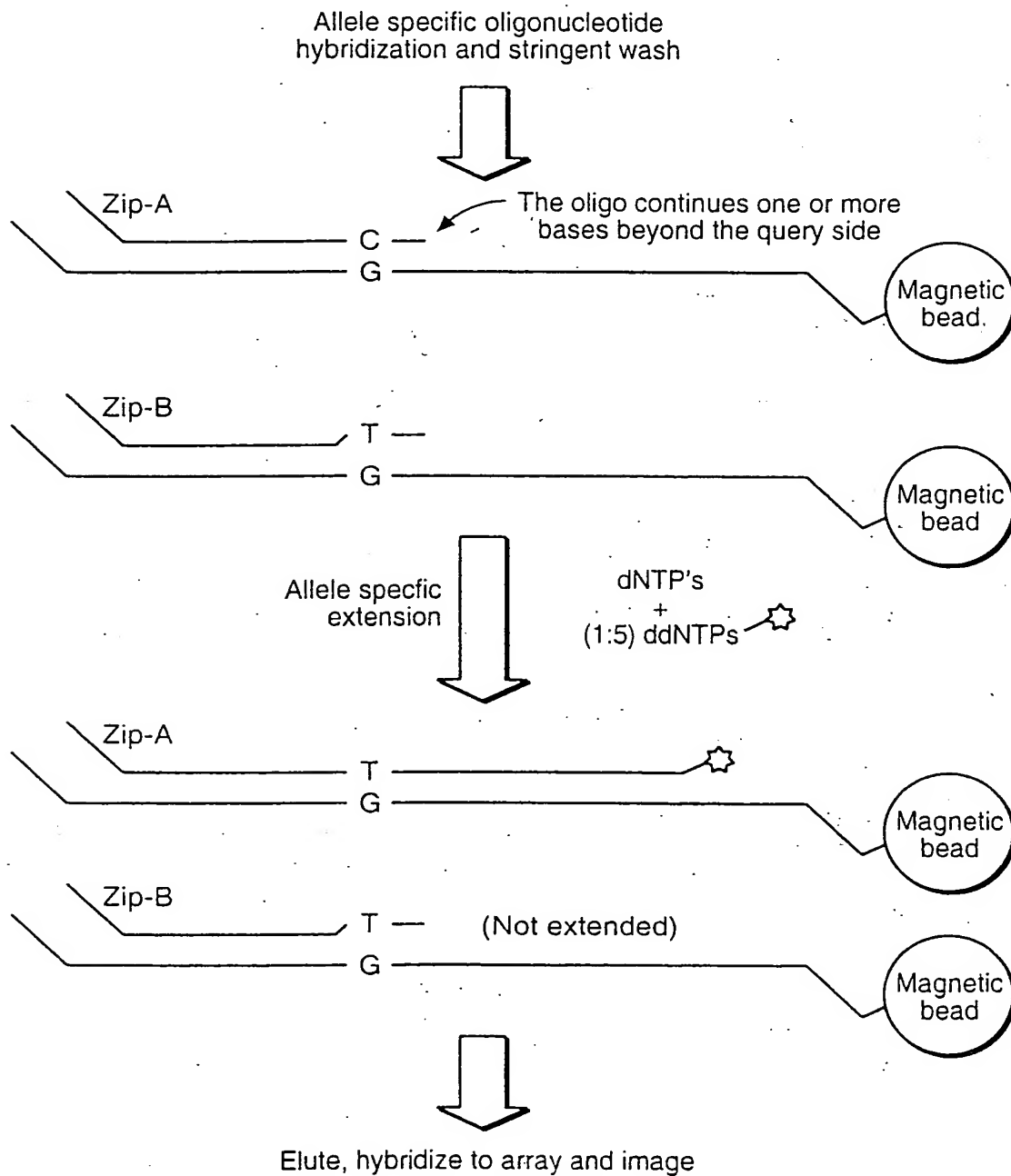


FIG. 7

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Alternate Labeling Scheme for Primer Extension (High Signal)

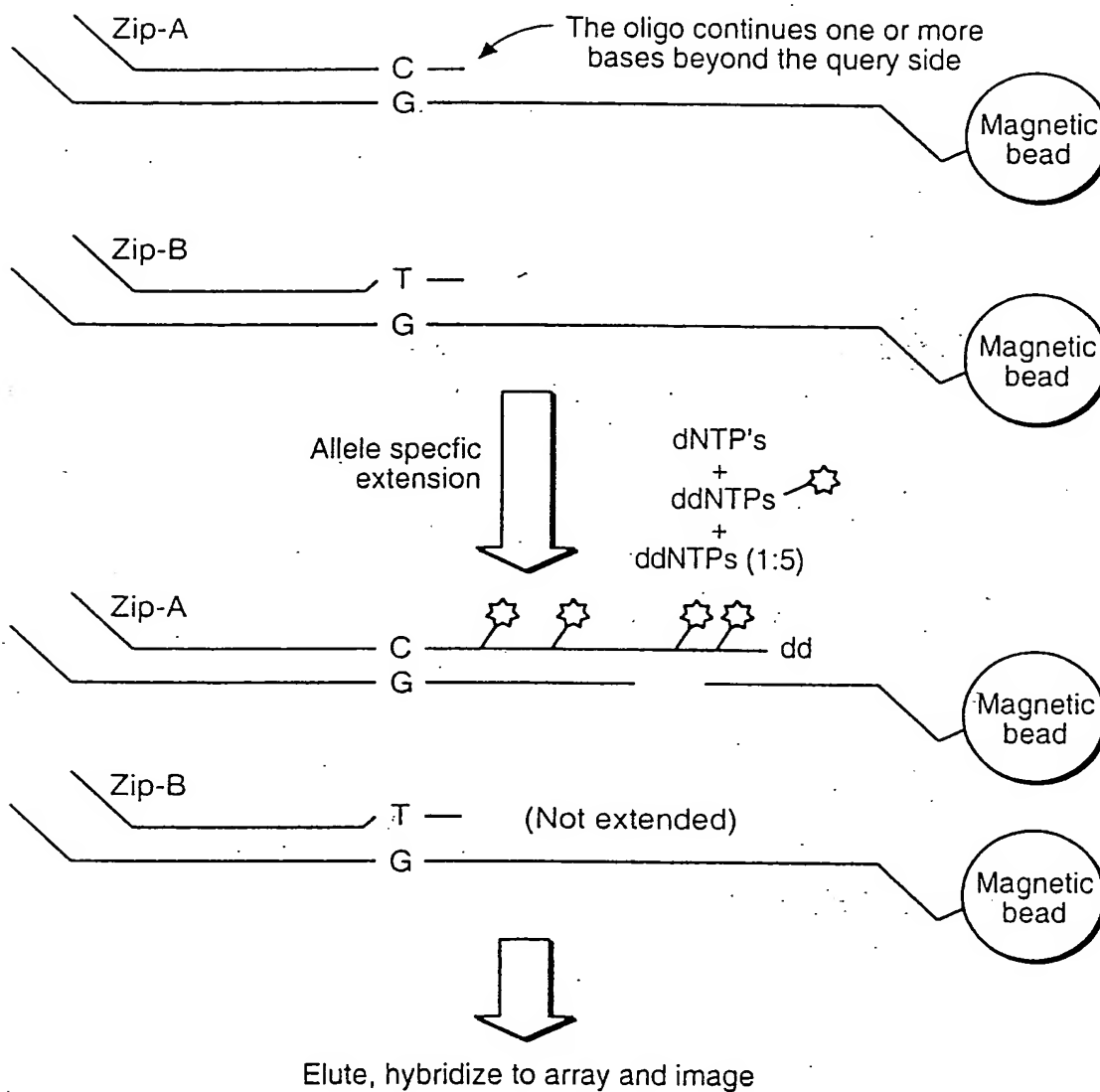


FIG. 8

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Simplified OLA-PCR Assay Format

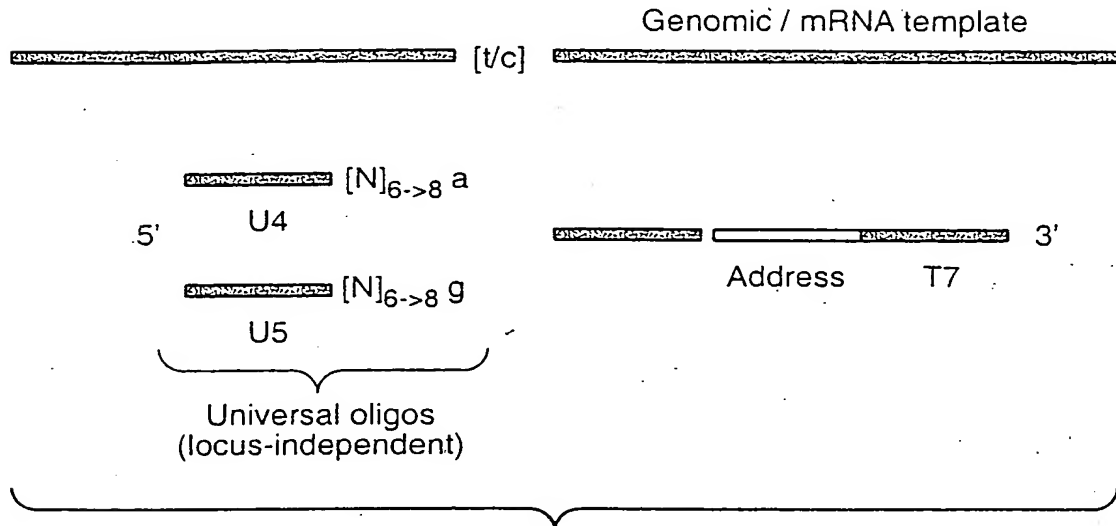


FIG. 9

"Reverse" S-OLA-PCR Assay Format

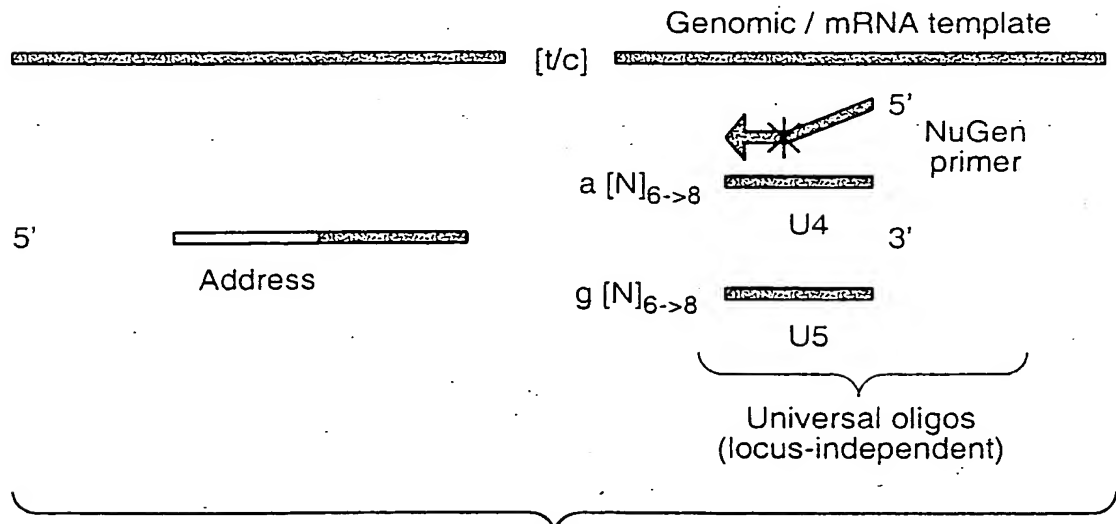


FIG. 10

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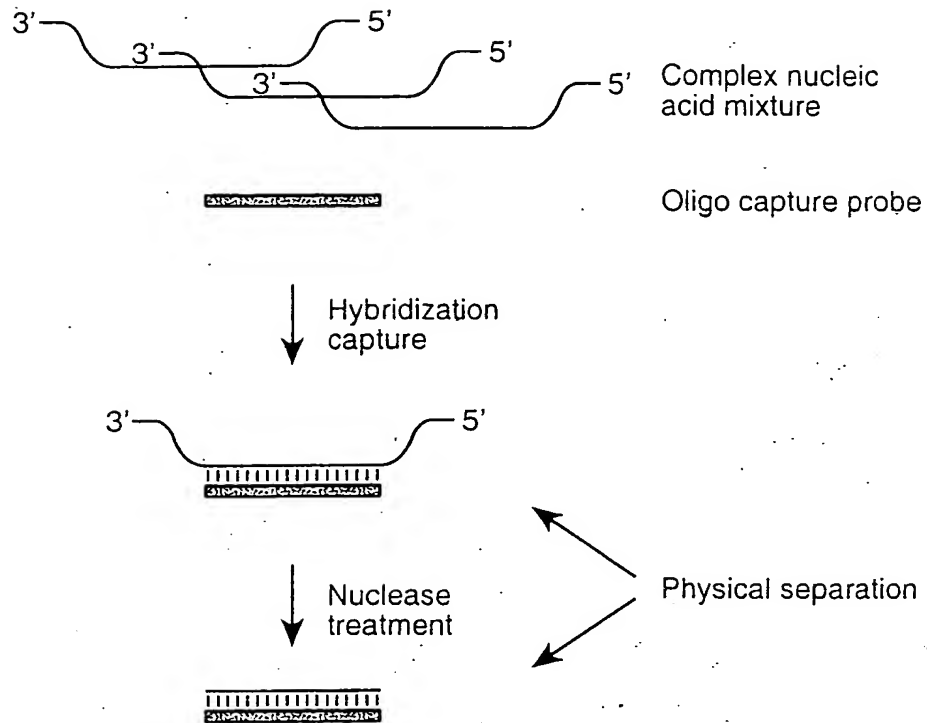


FIG. 11

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Principle of ICAN Method

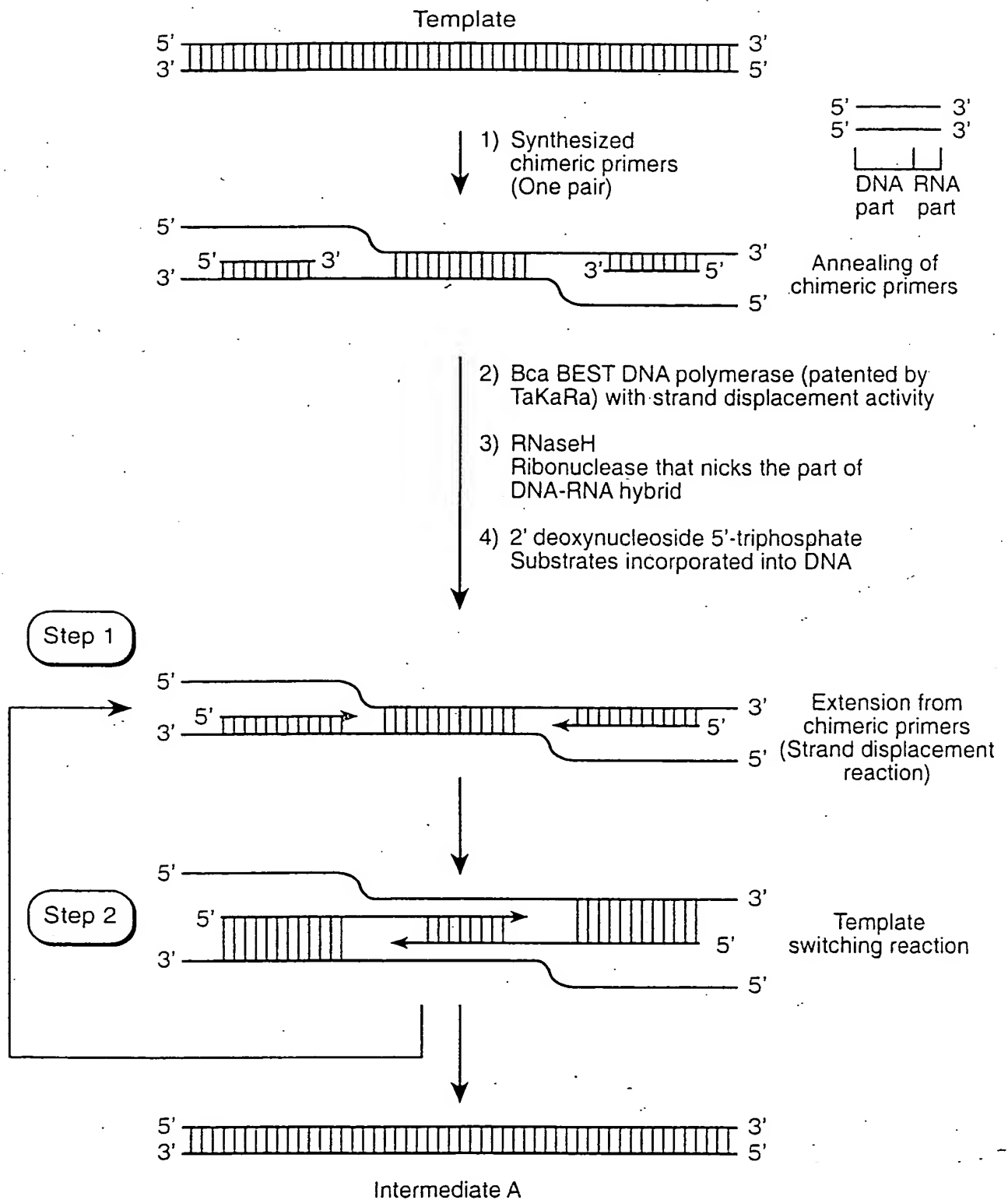


FIG. 12A

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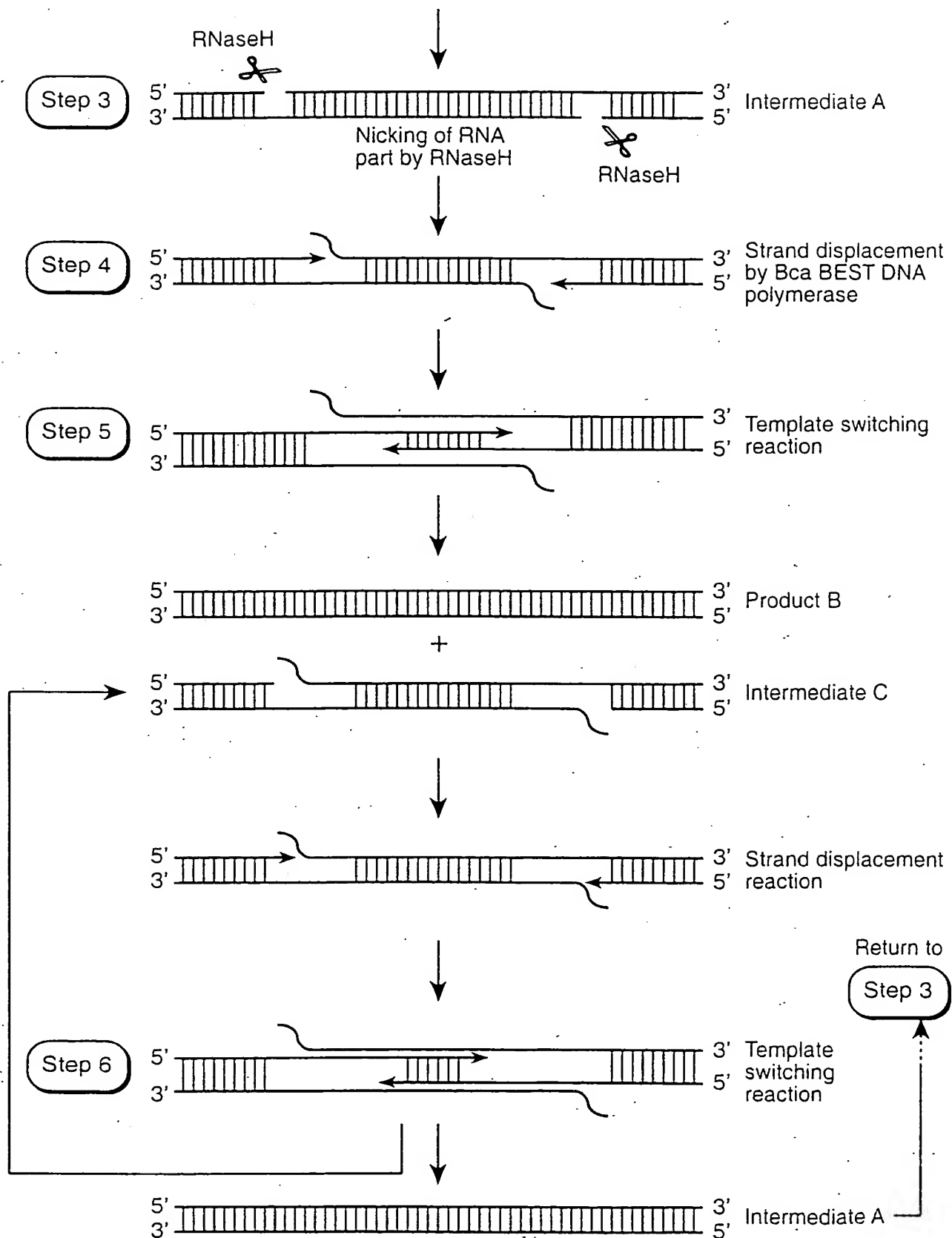


FIG. 12B

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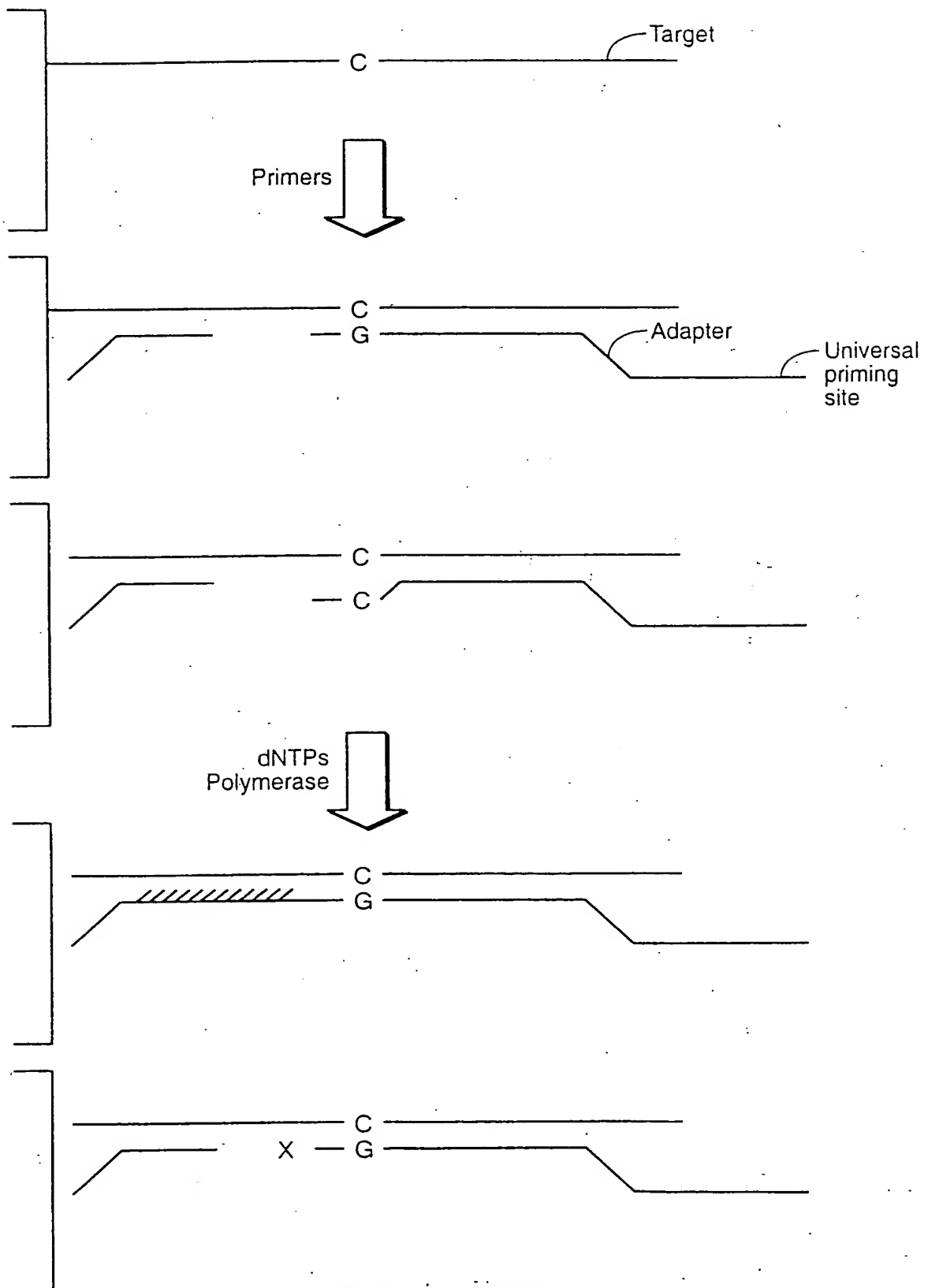


FIG. 13A

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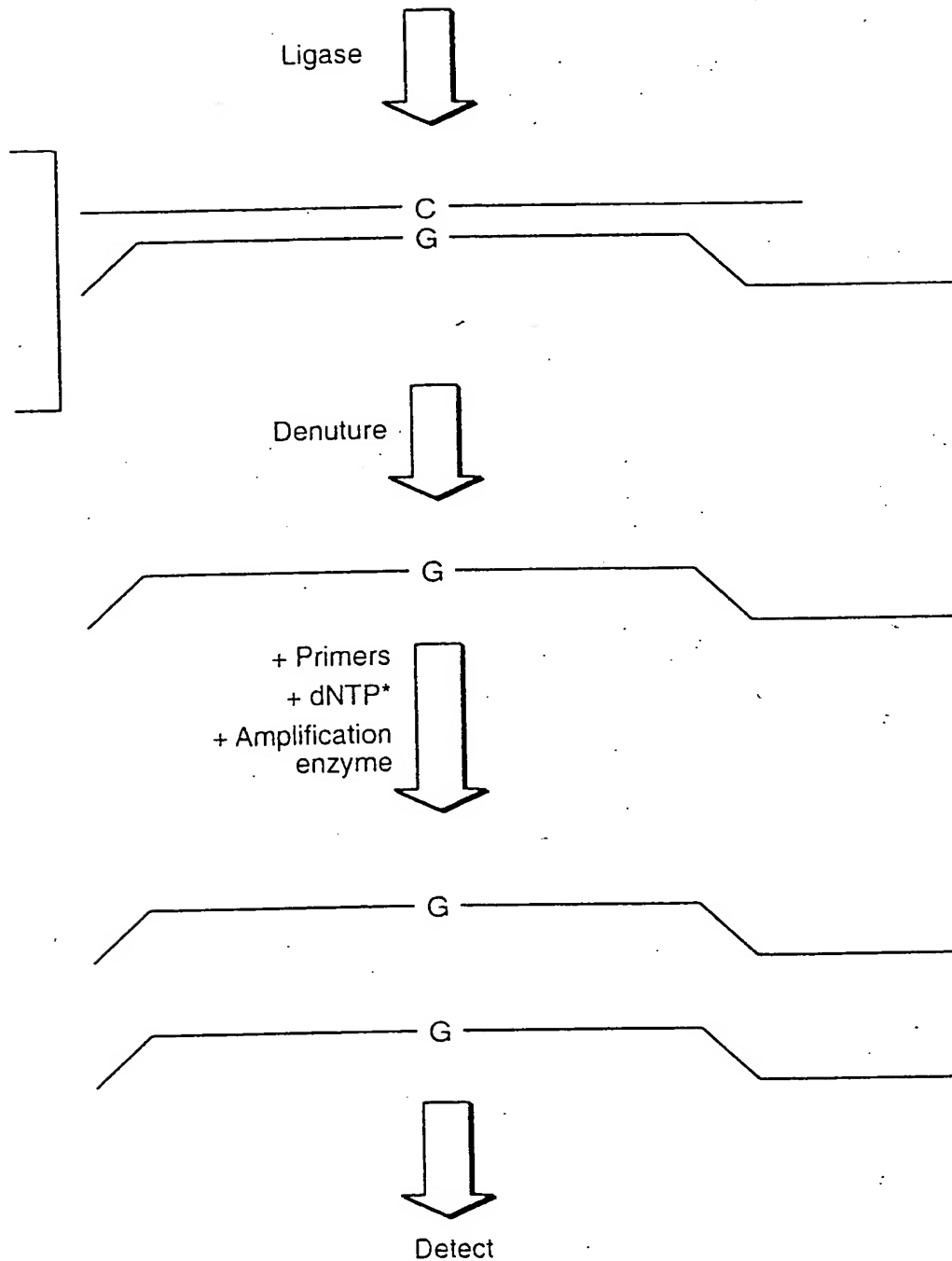


FIG._13B

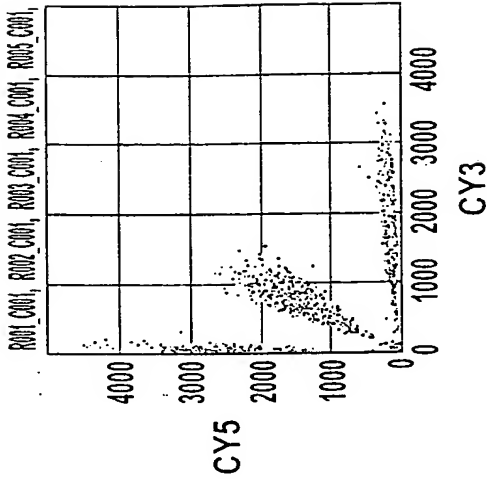


Fig. 14C

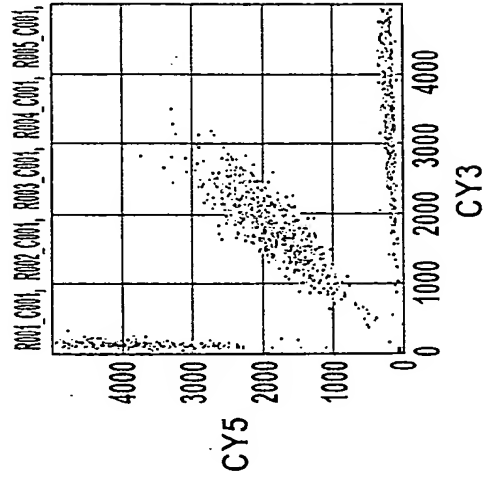


Fig. 14F

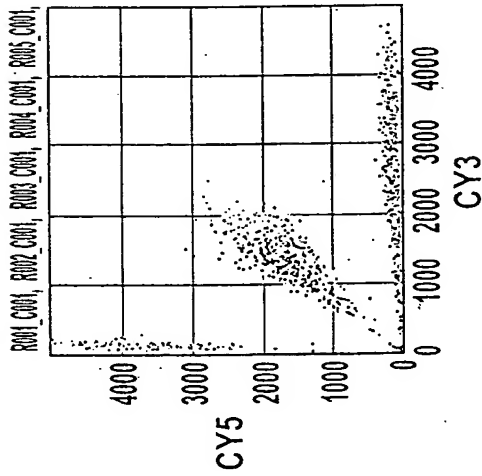


Fig. 14B

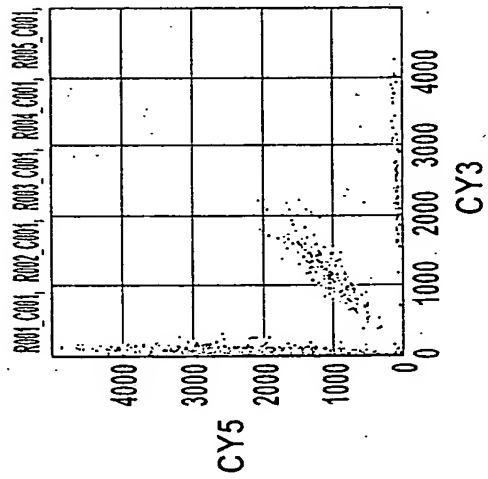


Fig. 14E

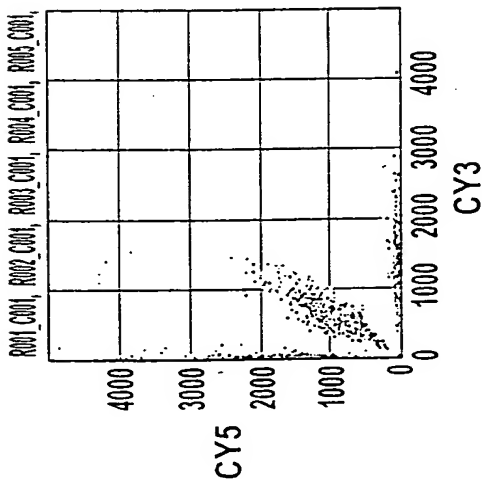


Fig. 14A

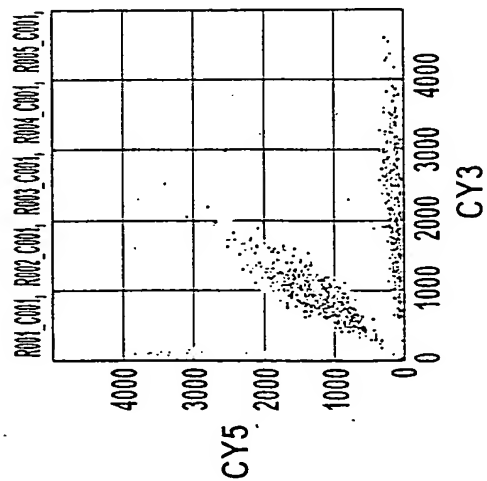


Fig. 14D

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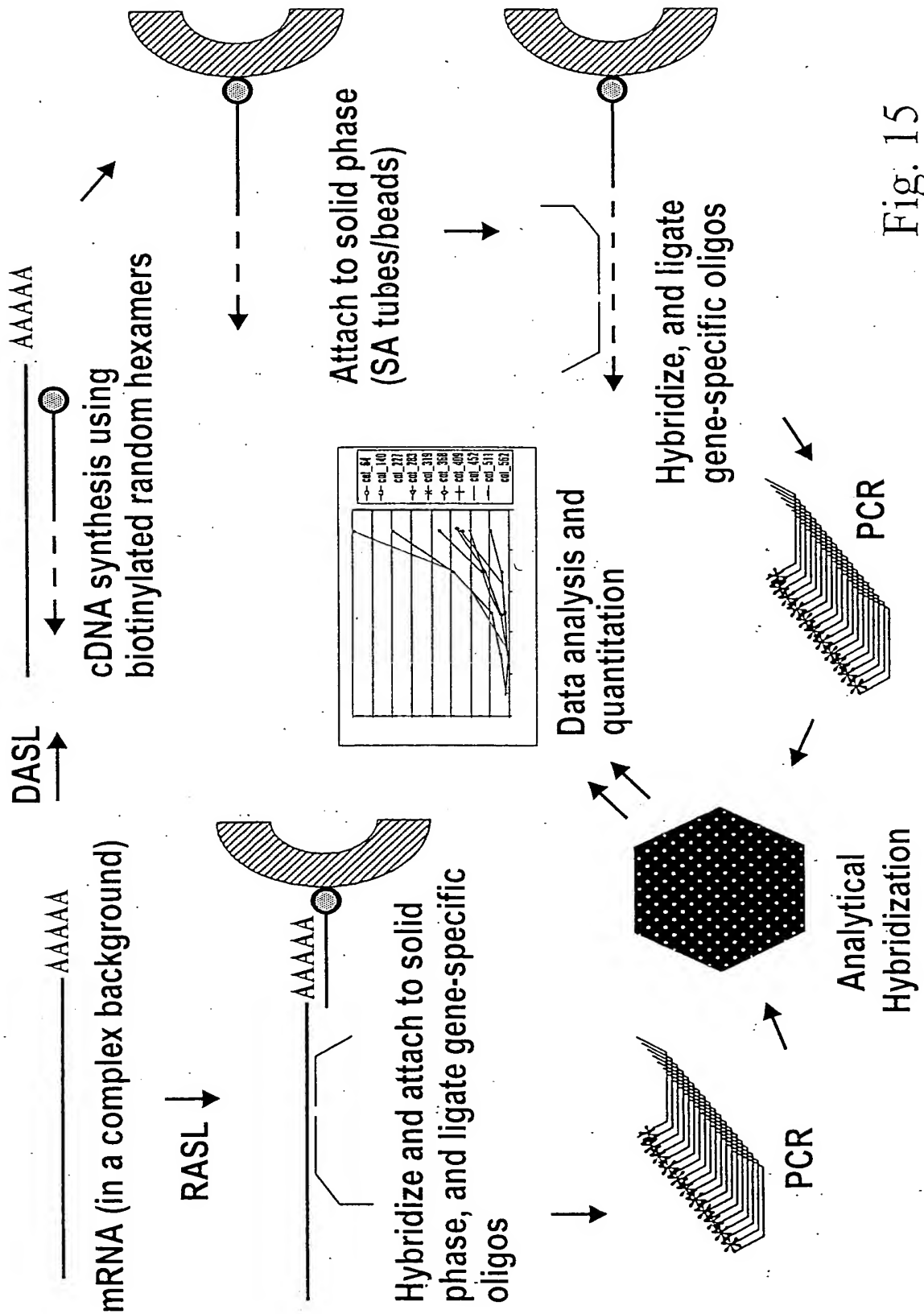
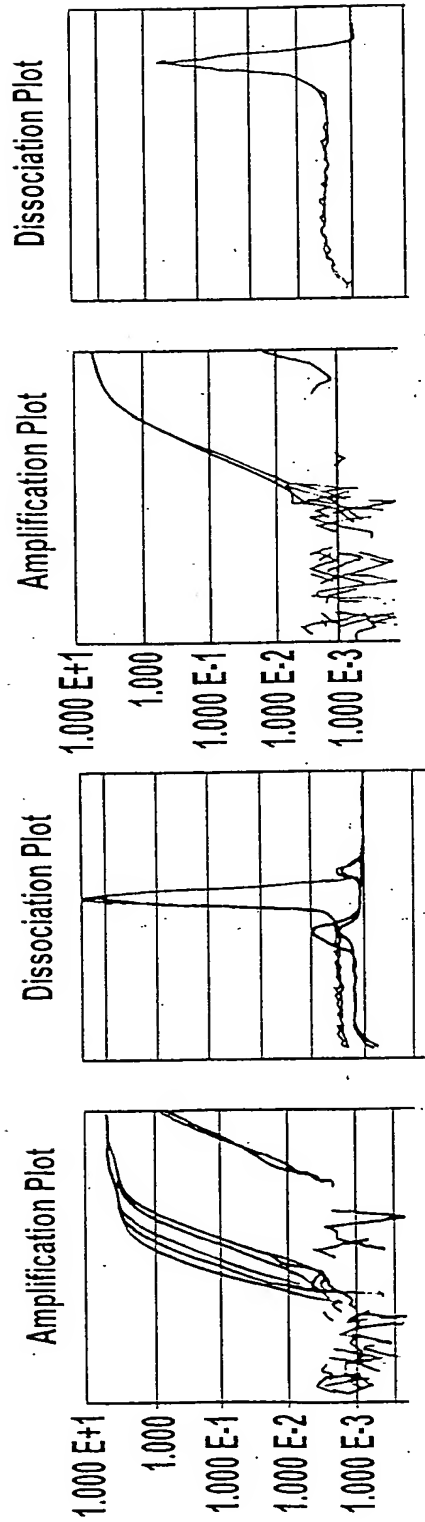


Fig. 15

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Mouse endogenous gene hsp74

Spike RNA - 550 bp fragment

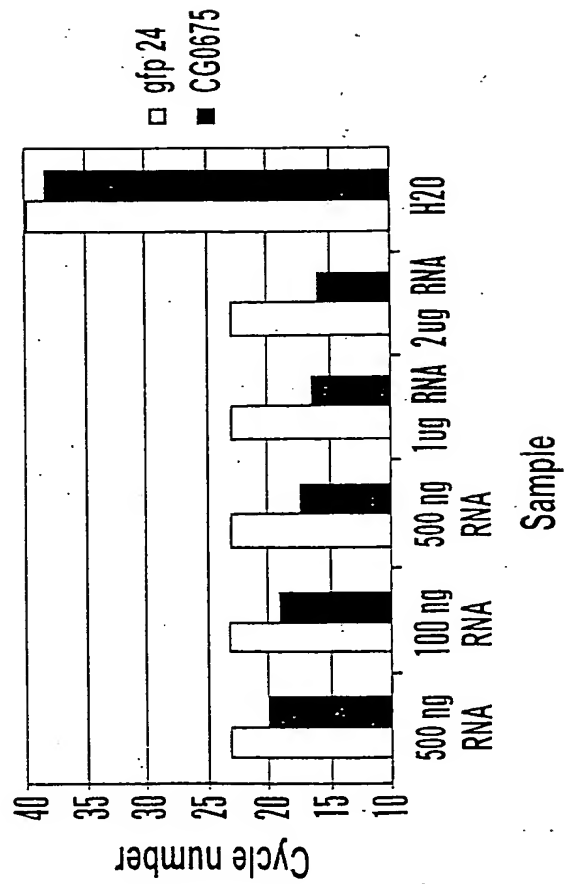
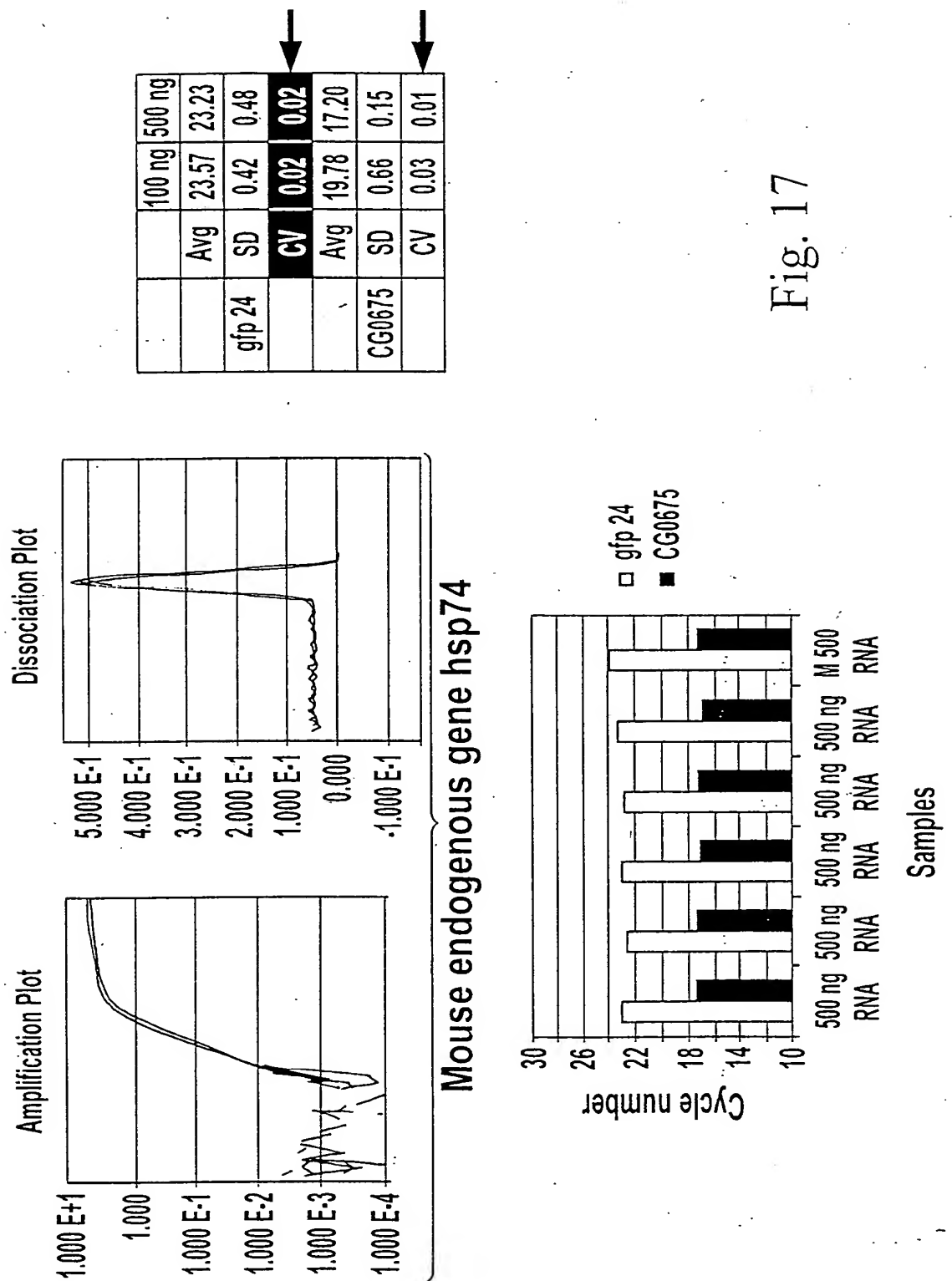


Fig. 16



	pool 1	pool 2	pool 3	pool 4	pool 5	pool 6	pool 7	pool 8
cat	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07
cre	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00
E1A	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04
GFP	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04
gus	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05
lacZ	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05
luc	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06
neo	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06
bla	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05
GST	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05



Fig. 18

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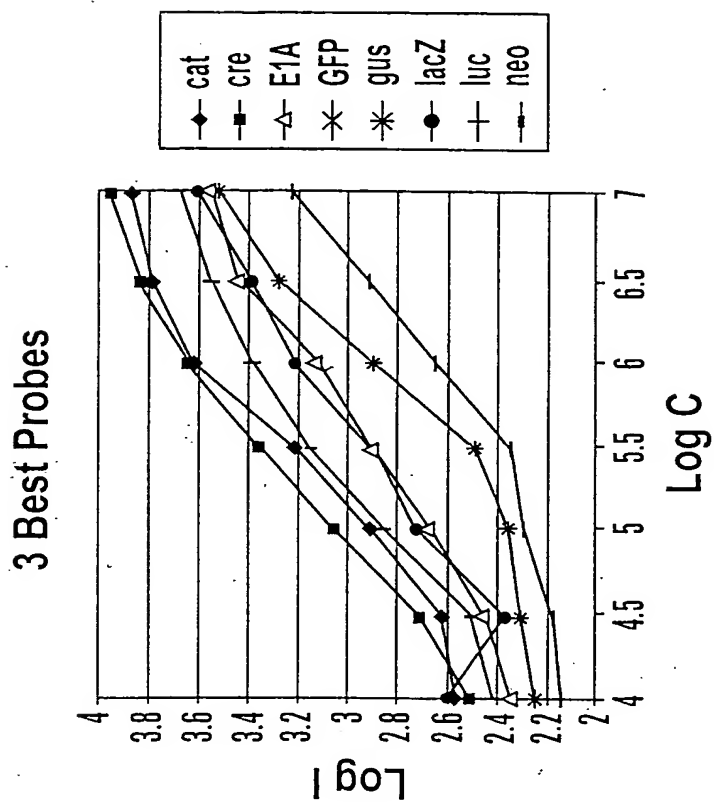


Fig. 19B

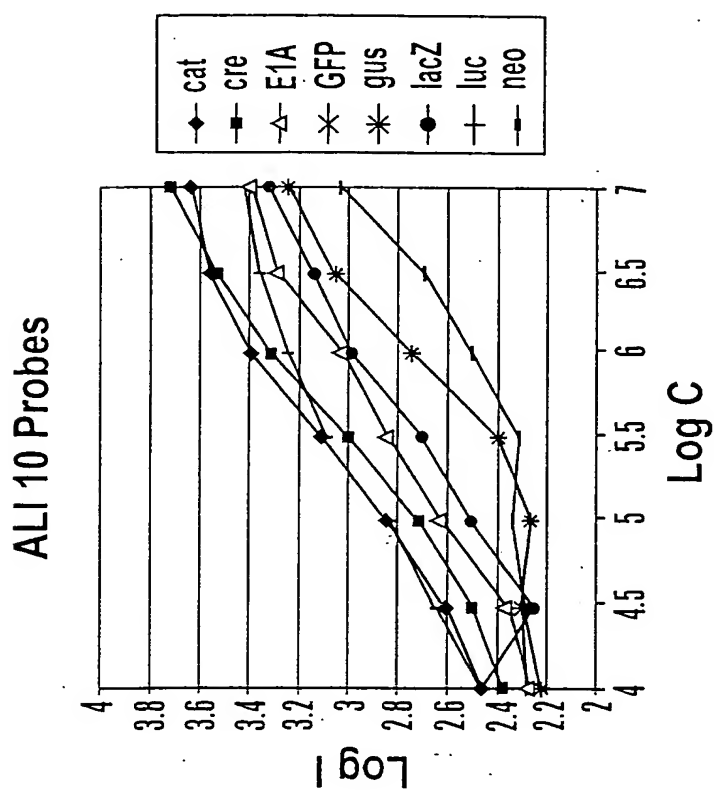
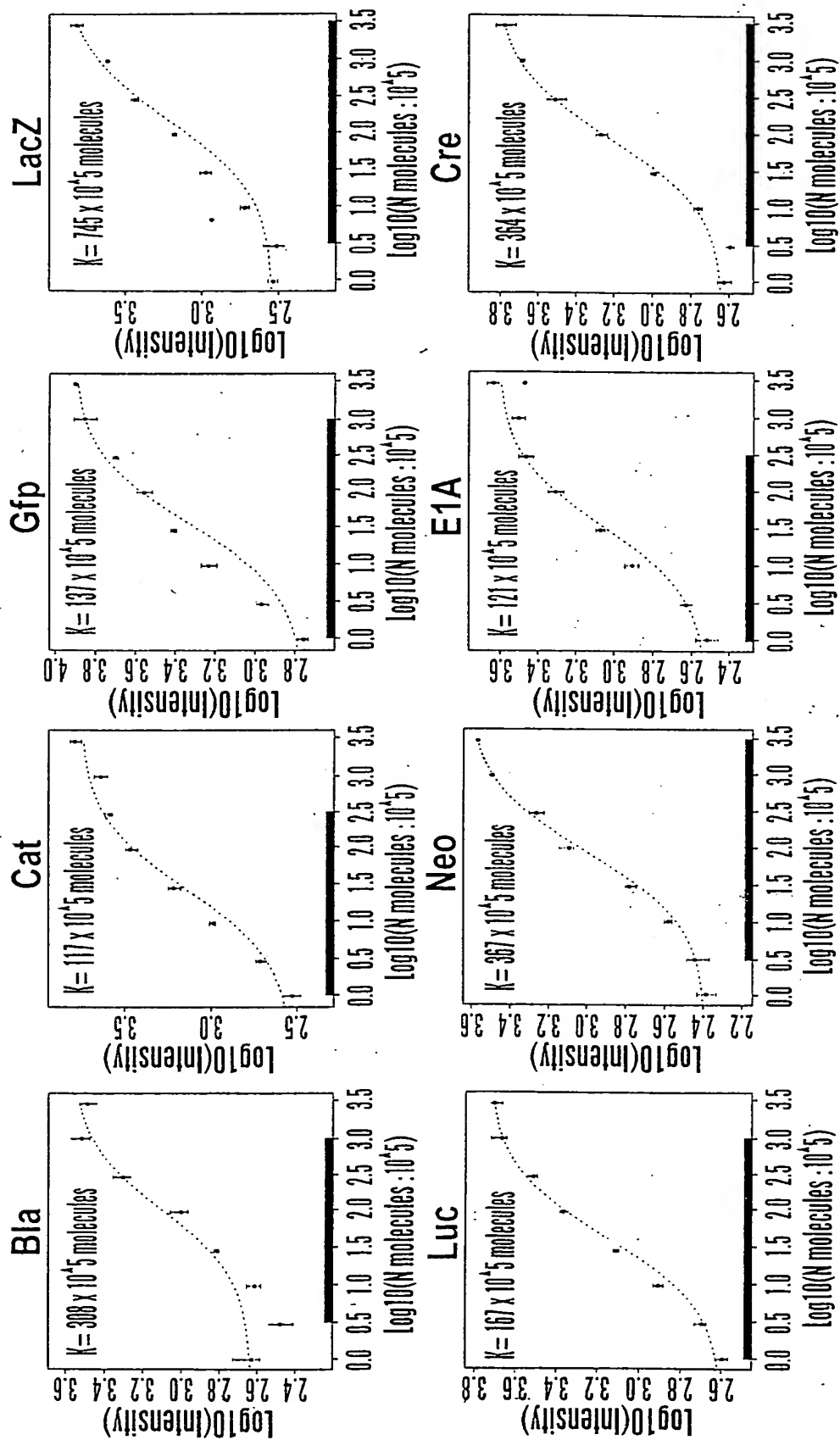


Fig. 19A

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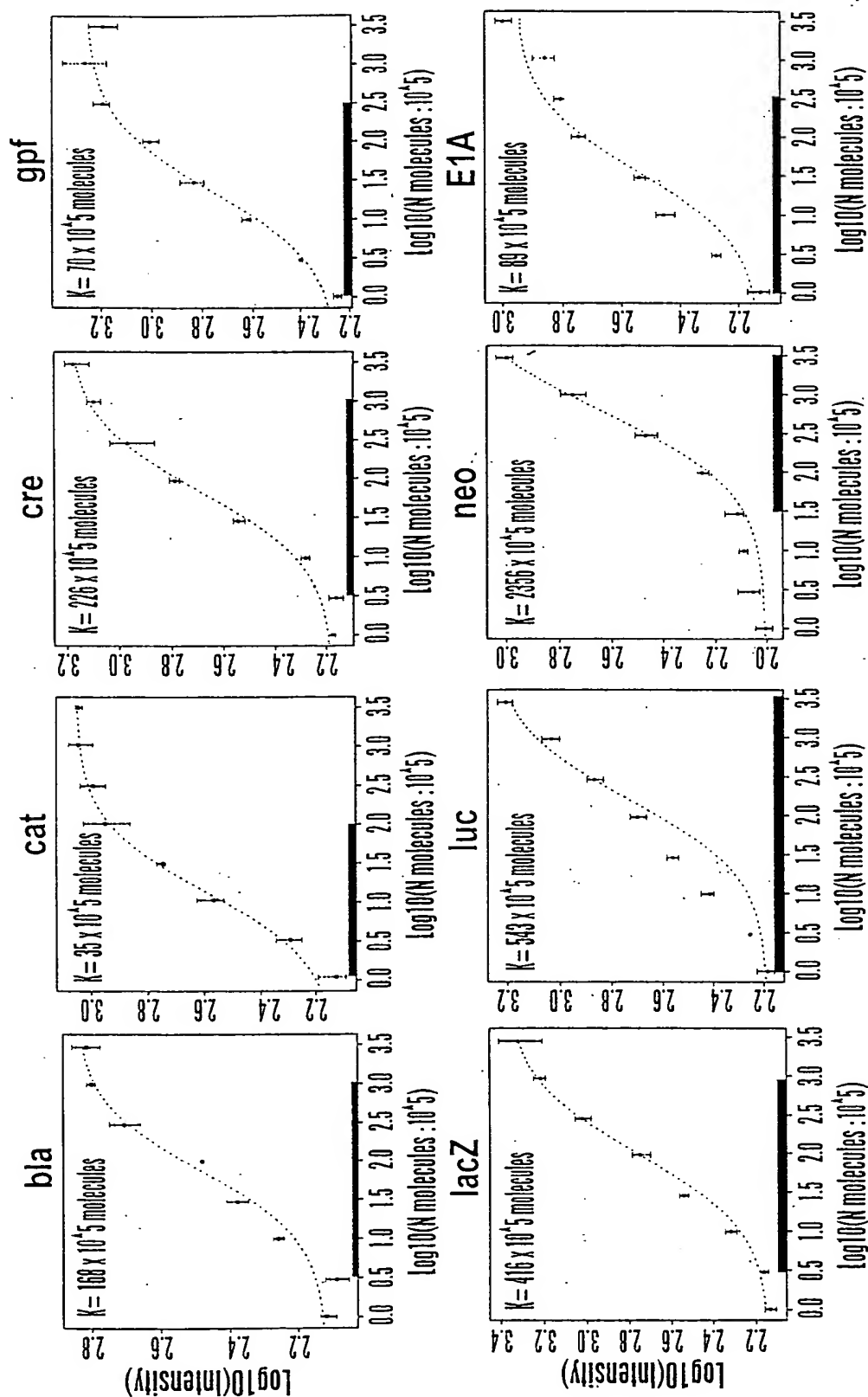


■ 3 fold detection range

• Error bars represent the range of intensities of 4 replicates.

Fig. 20

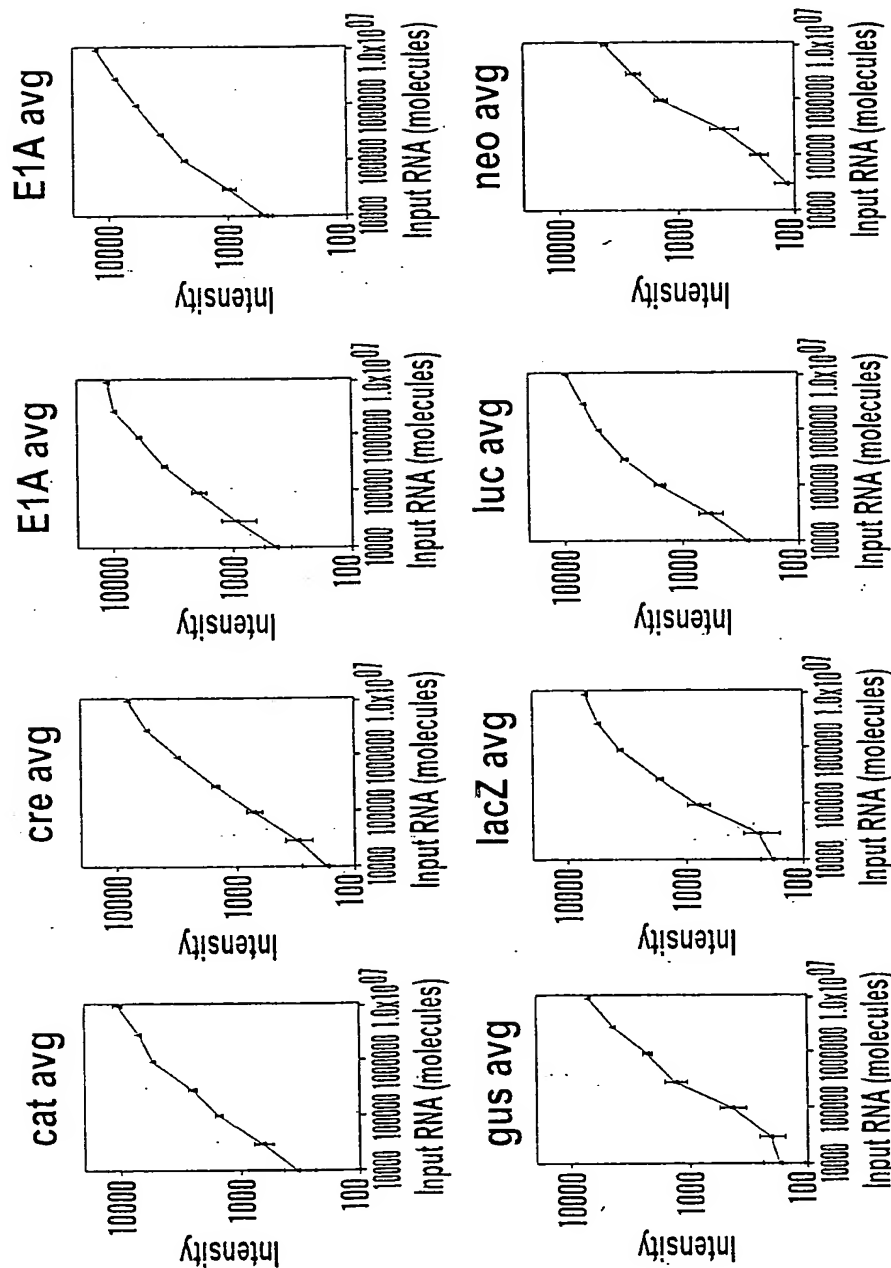
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- 250 ng of total RNA/sample
- Ds DNA hybridization
- Error bars represent the range of intensities of 4 replicates.

Fig. 21

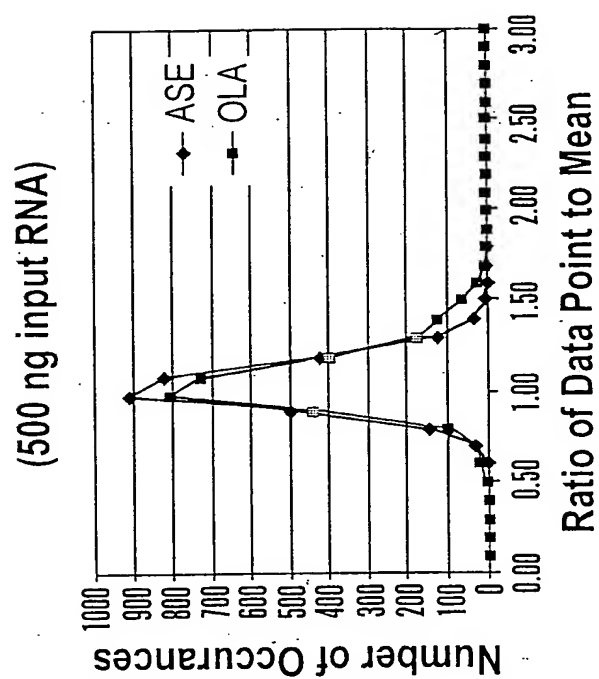
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- 100 ng total RNA background, 12 replicates, 238-plex.
- All pre-PCR and post-PCR processes identical to SciOps including single stranded product hybridization to arrays.
- Dynamic range: 2.5 - 3 logs; Precision: better than 3 fold change.

Fig. 22

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- 100.0% data points among 4 replicates within 2 fold change
- 98.8% data points among 4 replicates within 2 fold change

Fig. 23

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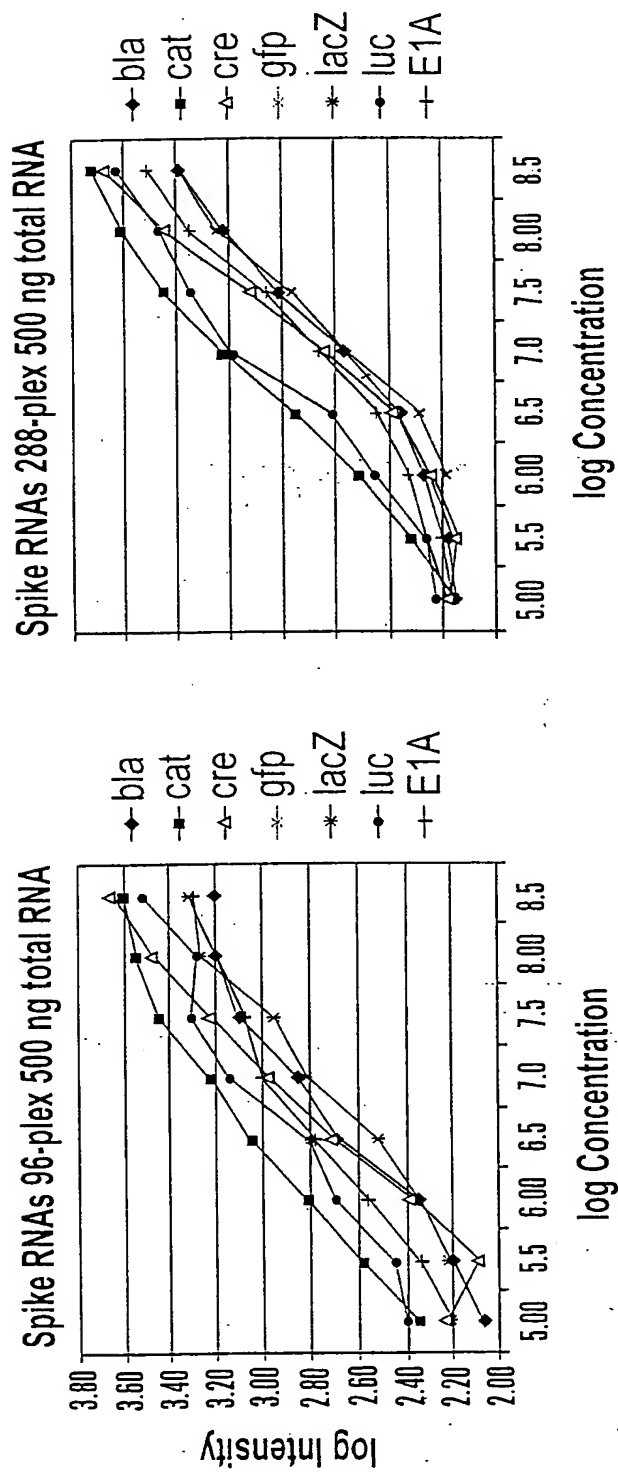
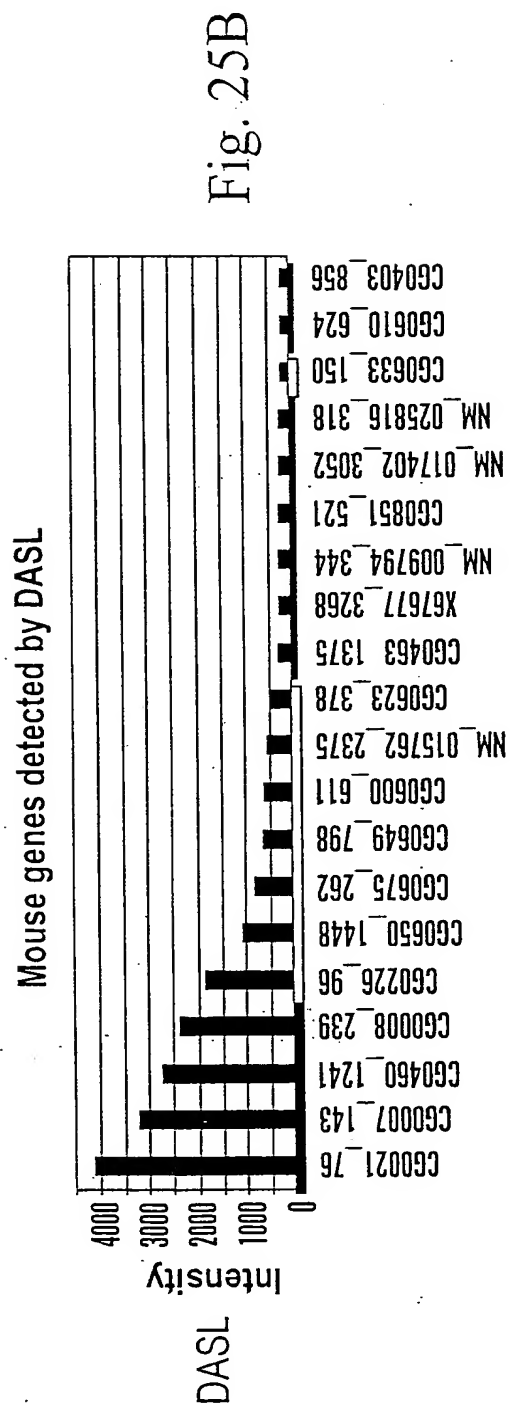
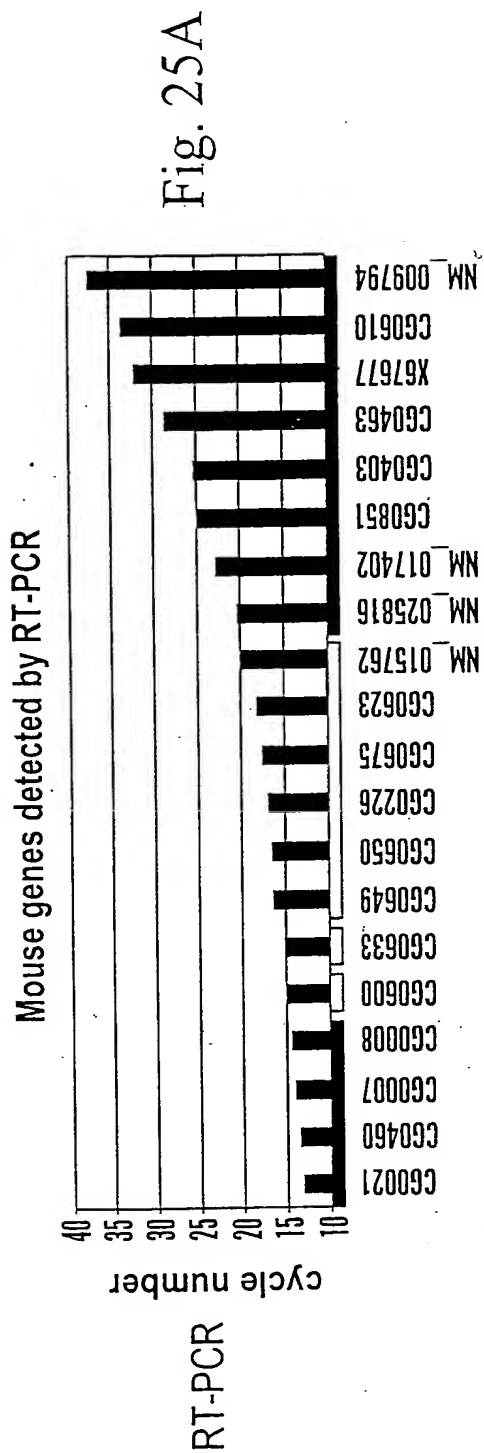


Fig. 24A

Fig. 24B

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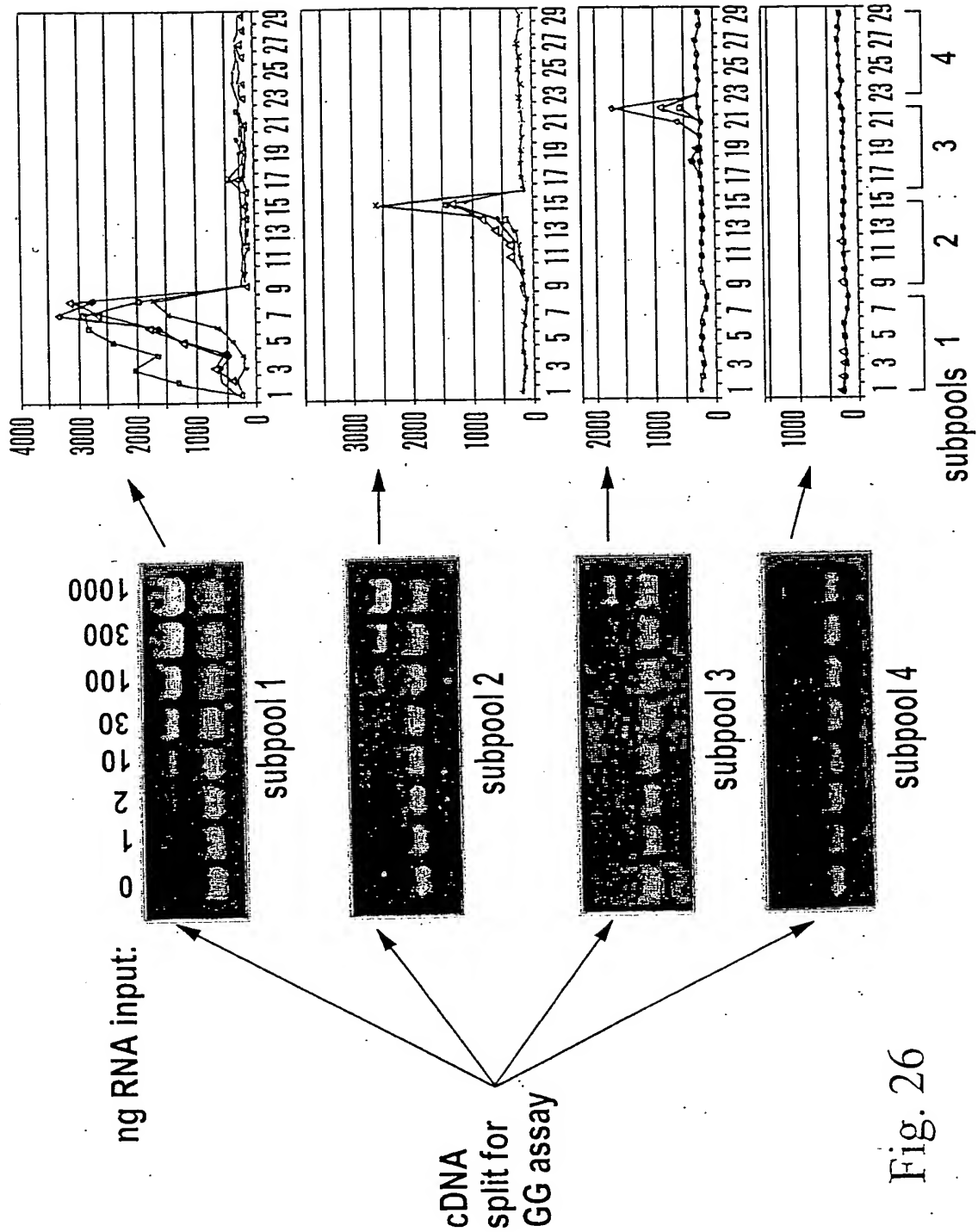


Fig. 26

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Fig. 27A

One pair of PCR primer

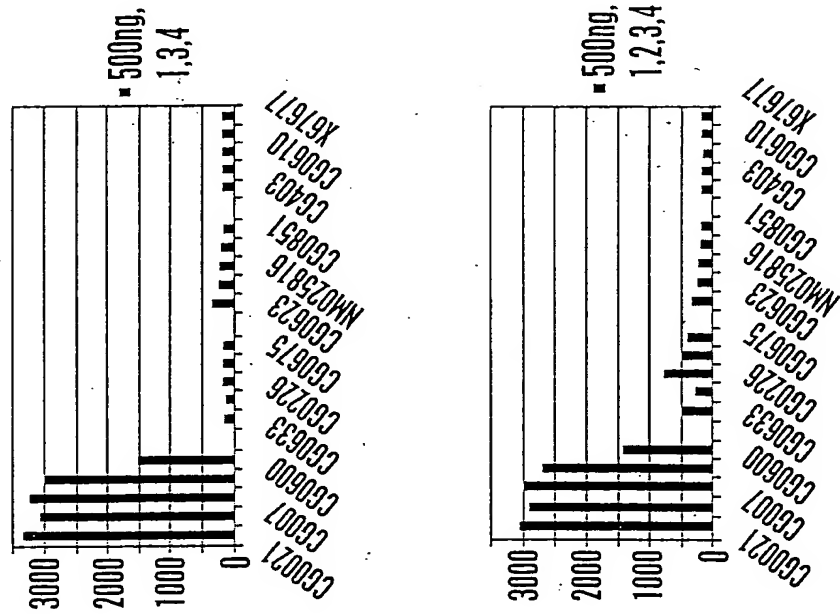


Fig. 27B

Two pairs of PCR primer

